

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

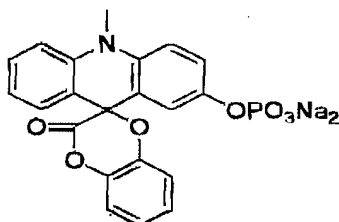
AMENDMENT TO THE CLAIMS

1-7. (Cancelled)

8. (Currently amended) The chemiluminescent substrate of claim 43 wherein said counter ions A are selected from the group consisting of  $\text{CH}_3\text{SO}_4^-$ ,  $\text{FSO}_3^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $\text{C}_4\text{F}_9\text{SO}_3^-$ ,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$ , halide,  $\text{CF}_3\text{COO}^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{NO}_3^-$ .

9-21. (Cancelled)

22. (Currently amended) The chemiluminescent substrate of claim 21 61 having the following structure:



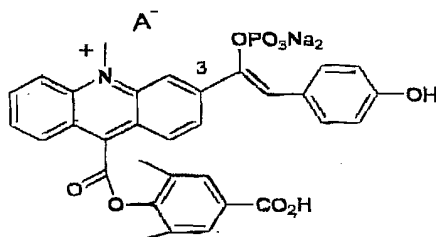
23-24. (Cancelled)

25. (Currently amended) The A chemiluminescent substrate of claim ~~23~~ having the following structure:

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651



wherein  $A^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said counter ion  $A^-$  is selected from the group consisting of  $CH_3SO_3^-$ ,  $FSO_3^-$ ,  $CF_3SO_3^-$ ,  $C_4F_9SO_3^-$ ,  $CH_3C_6H_4SO_3^-$ , halide,  $CF_3COO^-$ ,  $CH_3COO^-$ , and  $NO_3^-$ .

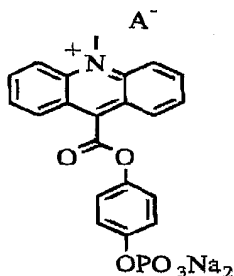
Application No. 09/626,366

Filed: July 27, 2000

Group Art Unit: 1651

26-28. (Cancelled)

29. (Currently amended) The ~~A~~ chemiluminescent substrate of ~~claim 26~~ having the following structure:

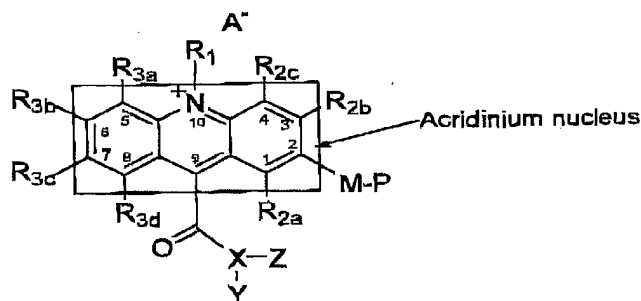


wherein  $A^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said counter ion  $A^-$  is selected from the group consisting of  $CH_3SO_4^-$ ,  $FSO_3^-$ ,  $CF_3SO_3^-$ ,  $C_4F_9SO_3^-$ ,  $CH_3C_6H_4SO_3^-$ , halide,  $CF_3COO^-$ ,  $CH_3COO^-$ , and  $NO_3^-$ .

30-42. (Cancelled)

43. (Currently amended) The ~~A~~ chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

Application No. 09/626,566  
 Filed: July 27, 2000  
 Group Art Unit: 1651



wherein

P is  $\text{PO}_3\text{Na}_2$  or a sugar moiety;

M is oxygen;

$R_1$  is selected from the group consisting of methyl, sulfopropyl and sulfobutyl;

$R_{2a}$ ,  $R_{2b}$ ,  $R_{2c}$ ,  $R_{3a}$ ,  $R_{3b}$ ,  $R_{3c}$  and  $R_{3d}$ , are hydrogen;

$A^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $A^-$  not being present if said  $R_1$  substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

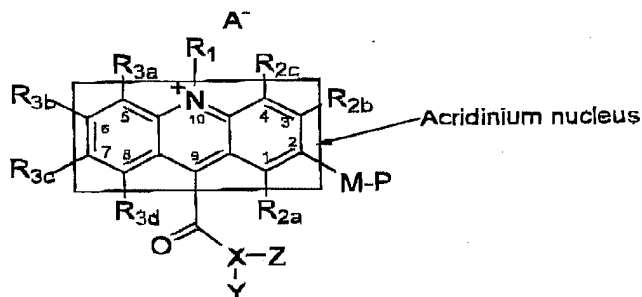
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

Application No. 09/626,566  
 Filed: July 27, 2000  
 Group Art Unit: 1651

44. (Currently amended) ~~The~~ A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure,



wherein

P is  $\text{PO}_3\text{Na}_2$  or a sugar moiety;

M is oxygen;

$\text{R}_1$  is selected from the group consisting of methyl, sulfopropyl and sulfobutyl;

$\text{R}_{2a}$ ,  $\text{R}_{2b}$ ,  $\text{R}_{2c}$ ,  $\text{R}_{3a}$ ,  $\text{R}_{3b}$ ,  $\text{R}_{3c}$  and  $\text{R}_{3d}$ , are hydrogen;

$\text{A}^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $\text{A}^-$  not being present if said  $\text{R}_1$  substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

X is O; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

45. (Previously added) The chemiluminescent substrate of claim 43, wherein

P is  $\text{PO}_3\text{Na}_2$ ;

X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

46. (Previously added) The chemiluminescent substrate of claim 43, wherein

P is  $\text{PO}_3\text{Na}_2$ ;

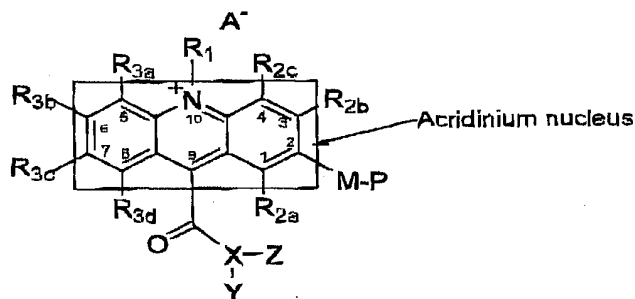
X is S; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

47. (New) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651



wherein

P is  $\text{PO}_3\text{Na}_2$  or a sugar moiety;

M is oxygen;

$\text{R}_1$  is selected from the group consisting of sulfoalkyl and carboxymethyl;

$\text{R}_{2a}$ ,  $\text{R}_{2b}$ ,  $\text{R}_{2c}$ ,  $\text{R}_{3a}$ ,  $\text{R}_{3b}$ ,  $\text{R}_{3c}$  and  $\text{R}_{3d}$ , can be the same or different, selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano ( $-\text{CN}$ );

$\text{A}^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $\text{A}^-$  not being present if said  $\text{R}_1$  substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-

Application No. 09/626,566

Filed: July 27, 2000

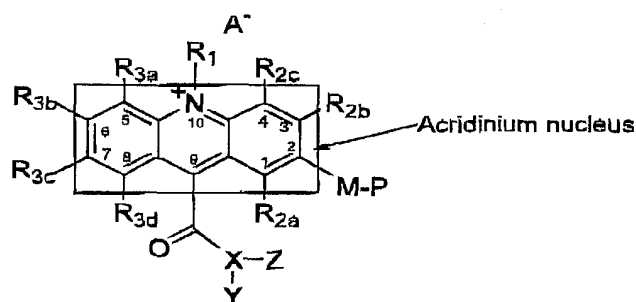
Group Art Unit: 1651

benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl,; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

48. (New) The chemiluminescent substrate of claim 47 wherein said counter ions  $A^-$  are selected from the group consisting of  $CH_3SO_4^-$ ,  $FSO_3^-$ ,  $CF_3SO_3^-$ ,  $C_4F_9SO_3^-$ ,  $CH_3C_6H_4SO_3^-$ , halide,  $CF_3COO^-$ ,  $CH_3COO^-$ , and  $NO_3^-$ .

49. (New) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein



Application No. 09/626,566  
Filed: July 27, 2000  
Group Art Unit: 1651

P is selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  $\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$  and  $\text{C}(=\text{O})\text{R}$  group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

$\text{R}_1$  is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

$\text{R}_{2a}$ ,  $\text{R}_{2b}$ ,  $\text{R}_{2c}$ ,  $\text{R}_{3a}$ ,  $\text{R}_{3b}$ ,  $\text{R}_{3c}$  and  $\text{R}_{3d}$ , can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, and cyano ( $-\text{CN}$ );

$\text{A}^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $\text{A}^-$  not being present if said  $\text{R}_1$  substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl,

Application No. 09/626,566

Filed: July 27, 2000

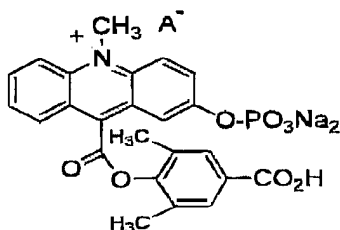
Group Art Unit: 1651

(2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl,; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

50. (New) The chemiluminescent substrate of claim 49 wherein said counter ions  $A^-$  are selected from the group consisting of  $CH_3SO_4^-$ ,  $FSO_3^-$ ,  $CF_3SO_3^-$ ,  $C_4F_9SO_3^-$ ,  $CH_3C_6H_4SO_3^-$ , halide,  $CF_3COO^-$ ,  $CH_3COO^-$ , and  $NO_3^-$ .

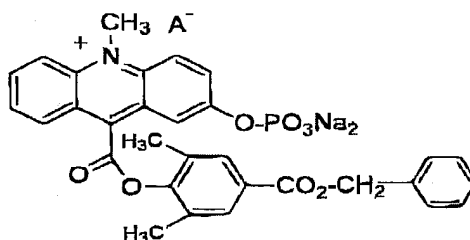
51. (New) The chemiluminescent substrate of Claim 43 having the structure,



wherein  $A^-$  is selected from the group consisting of  $CH_3SO_4^-$ ,  $FSO_3^-$ ,  $CF_3SO_3^-$ ,  $C_4F_9SO_3^-$ ,  $CH_3C_6H_4SO_3^-$ , halide,  $CF_3COO^-$ ,  $CH_3COO^-$ , and  $NO_3^-$ .

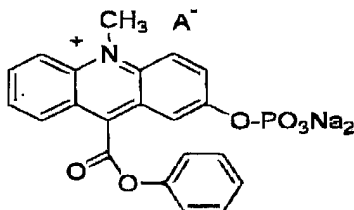
Application No. 09/626,566  
 Filed: July 27, 2000  
 Group Art Unit: 1651

52. (New) The chemiluminescent substrate of Claim 43 having the structure,



wherein A- is selected from the group consisting of  $\text{CH}_3\text{SO}_4^-$ ,  $\text{FSO}_3^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $\text{C}_4\text{F}_9\text{SO}_3^-$ ,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$ , halide,  $\text{CF}_3\text{COO}^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{NO}_3^-$ .

53. (New) The chemiluminescent substrate of Claim 43 having the structure,



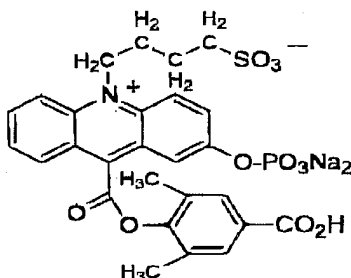
wherein A- is selected from the group consisting of  $\text{CH}_3\text{SO}_4^-$ ,  $\text{FSO}_3^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $\text{C}_4\text{F}_9\text{SO}_3^-$ ,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$ , halide,  $\text{CF}_3\text{COO}^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{NO}_3^-$ .

Application No. 09/626,566

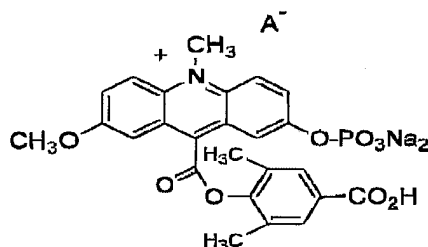
Filed: July 27, 2000

Group Art Unit: 1651

54. (New) The chemiluminescent substrate of Claim 43 having the structure



55. (New) The chemiluminescent substrate of Claim 47 having the structure,



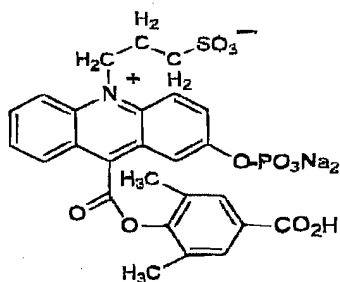
wherein A- is selected from the group consisting of  $\text{CH}_3\text{SO}_4^-$ ,  $\text{FSO}_3^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $\text{C}_4\text{F}_9\text{SO}_3^-$ ,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$ , halide,  $\text{CF}_3\text{COO}^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{NO}_3^-$ .

56. (New) The chemiluminescent substrate of Claim 43 having the structure

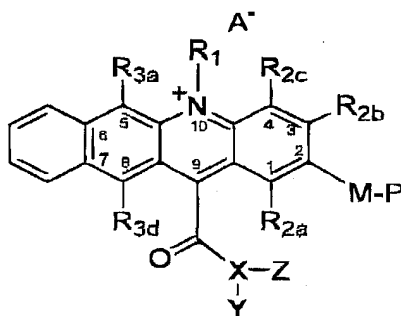
Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651



57. (New) A chemiluminescent substrate of having the structure



wherein

P is selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,

$\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and  $\text{C}(=\text{O})\text{R}$  group

wherein R is an alkyl group having 1 to 6 carbon atoms;

Application No. 09/626,566  
Filed: July 27, 2000  
Group Art Unit: 1651

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, and R<sub>3d</sub>, can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN), ;

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl,; and Z is omitted; and

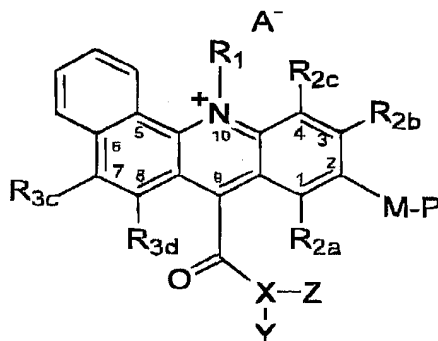
when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

58. (New) A chemiluminescent substrate having the structure



wherein

P is selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  $\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and  $\text{C}(=\text{O})\text{R}$  group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

$\text{R}_1$  is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

$\text{R}_{2a}$ ,  $\text{R}_{2b}$ ,  $\text{R}_{2c}$ ,  $\text{R}_{3c}$  and  $\text{R}_{3d}$ , can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, and cyano ( $-\text{CN}$ );

$\text{A}^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $\text{A}^-$  not being





Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

wherein

P is selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  $\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and  $\text{C}(=\text{O})\text{R}$  group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

$\text{R}_1$  is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

$\text{R}_{2a}$ ,  $\text{R}_{2b}$ ,  $\text{R}_{2c}$ ,  $\text{R}_{3a}$ , and  $\text{R}_{3b}$  can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano ( $-\text{CN}$ ), ;

$\text{A}^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $\text{A}^-$  not being present if said  $\text{R}_1$  substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-

-20-

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Application No. 09/626,566

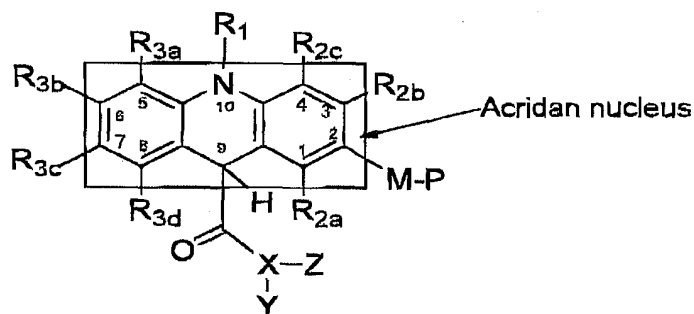
Filed: July 27, 2000

Group Art Unit: 1651

benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl,  
 (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-  
 carboxyl)phenyl,; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

60. (New) A chemiluminescent substrate of a hydrolytic enzyme,  
 said substrate having the structure



wherein

P is selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  $\text{PO}_3\text{K}_2$ ,  
 $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and  $\text{C}(=\text{O})\text{R}$  group  
 wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, R<sub>3b</sub>, R<sub>3c</sub> and R<sub>3d</sub>, can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN), ;

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl,; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

The chemical structure shows a macrocyclic compound. It features a central nitrogen atom (N10) and a carbonyl group (C=O). The structure is composed of two benzene rings and a central ring system. The substituents are labeled as R1, R2a, R2b, R2c, R3a, R3b, R3c, R3d, and R11. The atoms in the central ring are labeled X1, X2, Z1, and Z2. The numbering 1-11 is shown on the rings.

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group

Application No. 09/626,566

Filed: July 27, 2000

Group Art Unit: 1651

that can form an anion and pair with the quaternary ammonium cationic moiety; and

$X_1$  and  $X_2$  are the same or different and are selected from the group consisting of O, N or S, such that,

when  $X_1$  and  $X_2$  are O or S,  $R_{11}$  is selected from the group consisting of hydrogen, -R, substituted or unsubstituted aryl, halides, nitro, sulfonate, sulfate, phosphonate,  $-CO_2H$ ,  $-C(O)OR$ , cyano ( $-CN$ ),  $-SCN$ ,  $-OR$ ,  $-SR$ ,  $-SSR$ ,  $-C(O)R$ ,  $-C(O)NHR$ , ethylene glycol, or polyethylene glycol, where R is as defined above; and  $Z_1$  and  $Z_2$  are omitted; and

when at least one of  $X_1$  and  $X_2$  is N,  $Z_1$  and  $Z_2$  are toluenesulfonyl, and  $R_{11}$  is carboxypropyl.